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First Chromosome Counts for Four Families of Malpighiales

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The order Malpighiales, which comprises 39–40 families of flowering plants, are poorly understood in terms of their morphological characters and the relationships among the families. Here, we present the first chromosome counts of Irvingiaceae, Ixonanthaceae, Lacistemataceae, and Peraceae, based on one or two species each, using somatic cells from young leaves. The chromosome numbers were $2n = 30$ for *Irvingia malayana* (Irvingiaceae), $2n = 28$ for *Ixonanthes icosandra* and *I. reticulata* (Ixonanthaceae), $2n = 44$ for *Lacistema aggregatum* (Lacistemataceae), and $2n = 36$ for *Chaetocarpus castanocarpus* (Peraceae). The relationships of individual families based on chromosome numbers are discussed briefly.

Keywords: chromosome number, Irvingiaceae, Ixonanthaceae, Lacistemataceae, Malpighiales, Peraceae

The order Malpighiales comprises 39 to 40 families (Stevens 2001 onwards, APGIII 2009, Haston *et al.* 2009), among which the phylogenetic relationships remain unclear (Soltis *et al.* 2007, Wurdack & Davis 2009). Morphological and molecular analyses are needed to better understand the relationships of individual families. In terms of chromosome numbers, which often reflect the evolutionary relationships among families (e.g., Oginuma & Tobe 2006: Laurales) or within families (e.g., Oginuma & Tobe 1997: Rhizophoraceae), the Malpighiales are poorly studied. Of the 39 to 40 malpighialean families, the chromosome numbers of 12 remain unknown: Centroplacaceae, Ctenolophonaceae, Euphroniaceae, Goupiaceae, Irvingiaceae, Ixonanthaceae, Lacistemataceae, Lophopyxidaceae, Malesherbiaceae, Medusagynaceae, Peraceae, and Quiinaceae (Stevens 2001 onwards). Here, we report the first chromosome counts of four families: Irvingiaceae, Ixonanthaceae, Lacistemataceae, and Peraceae.

Materials and Methods

The species studied are listed in Table 1, along with collection data and chromosome numbers. Somatic chromosomes were examined using young leaves collected from trees growing in the wild or in botanical gardens. The methods of pretreatment, fixation, and staining for chromosome observations followed Oginuma *et al.* (1992). At least three to five cells were examined to determine the chromosome numbers.

The terminology of chromosome morphology based on the centromere position follows Levan *et al.* (1964). We examined all published data on the Malpighiales using Chromosome Numbers of Flowering Plants by Fedorov (1974), and serial publications titled Index to Plant Chromosome Numbers (Ornduff 1967–1968, Moore 1973–1977, Goldblatt 1981–1988, Goldblatt & Johnson 1990–2003). Information on chromosome numbers of individual families in the following discussion was obtained from those publications unless otherwise stated.

TABLE 1. Taxa of Irvingiaceae, Ixonanthaceae, Lacistemataceae and Peraceae (Malpighiales) studied, voucher information and chromosome numbers

Taxon	Collection	Chromosome number
Irvingiaceae		
Irvingia malayana Oliv.	SINGAPORE. Bukit Timah, *Oginuma & Lum s.n. in 2008* (KYO).	$2n = 30$
Ixonanthaceae		
Ixonanthes icosandra Jack	SINGAPORE. Bukit Timah, *Tobe & al. 1277* (KYO).	$2n = 28$
I. reticulata Jack	SINGAPORE. MacRitchie, *Tobe & al. 1279* (KYO).	$2n = 28$
Lacistemataceae		
Lacistema aggregatum Rusby	MEXICO. Chajul, Chiapas, *Tobe & Oginuma 349* (KYO).	$2n = 44$
Peraceae		
Chaetocarpus castanocarpus Thwaites	THAILAND. Khao Yai National Park, *Oginuma s.n.* in 2005 (KYO)	$2n = 36$

Results and Discussion

Irvingiaceae

The Irvingiaceae comprise eight species in the three genera *Desbordesia* Pierre ex Tiegh., *Irvingia* Hook.f. and *Klainedoxa* Pierre ex Engl., which are distributed from Africa to Southeast Asia in the Old World tropics (Takhtajan 2009). We studied *I. malayana* Oliv., one of four species in its genus, which had $2n = 30$. The chromosomes at metaphase gradually varied in length from about 0.7 to 1.4 μm (Fig. 1A, B). Of the 30 chromosomes, most had a median or submedian centromere, although the centromere position in several small chromosomes was unclear.

The base number of *Irvingia* is likely $x = 15$. Previous molecular analyses have suggested that Irvingiaceae are related to Linaceae (Davis *et al.* 2005, Tokuoka & Tobe 2006, Soltis *et al.* 2007, Korotkova *et al.* 2009), and to Caryocaraceae, Erythroxylaceae, and Rhizophoraceae (Soltis *et al.* 2007). While the base number reported here for *Irvingia* is substantially different from those of Caryocaraceae ($n = 23$), and Erythroxylaceae ($n = 12$), and for Rhizophoraceae ($x = 14$ or 16), a base number of $x = 15$ has been reported for a few species of *Linum* (Linaceae) and *Microdesmis* (Pandaceae). The base number ($x = 15$) common to *Irvingia*, *Linum* and *Microdesmis* may suggest relationships among the families to which those genera are assigned. It appears worthwhile to pay more attention to this possibility in future chromosome studies.

Ixonanthaceae

The Ixonanthaceae comprise 35 species in the five genera *Allanthospermum* Forman, *Cyrillopsis* Kuhlm., *Ixonanthes* Jack, *Ochthocosmus* Benth., and *Phyllocosmus* Klotzsch, all of which have pantropical distributions (Takhtajan 2009). We studied two species of *Ixonanthes*: *I. icosandra* Jack and *I. reticulata* Jack, both of which had $2n = 28$. The chromosomes at metaphase gradually varied in length from about 0.6 to 1.1 μm in *I. icosandra* (Fig. 1C, D) and from about 0.4 to 0.8 μm in *I. reticulata* (Fig. 1E, F). Of the 28 chromosomes, many had a centromere at a median or submedian position, although the centromere position in several chromosomes in both species was uncertain.

The base number of *Ixonanthes* is likely $x = 14$. The inter-family relationships of the Ixonanthaceae are uncertain, but molecular analyses have suggested a weak relationship with Ochnaceae (Tokuoka & Tobe 2006). Ochnaceae, however, likely have a base number of $x = 10$ (Oginuma unpublished). A base number of $x = 14$ is rare in other Malpighiales, but occurs in some Rhizophoraceae (Oginuma & Tobe 1997).

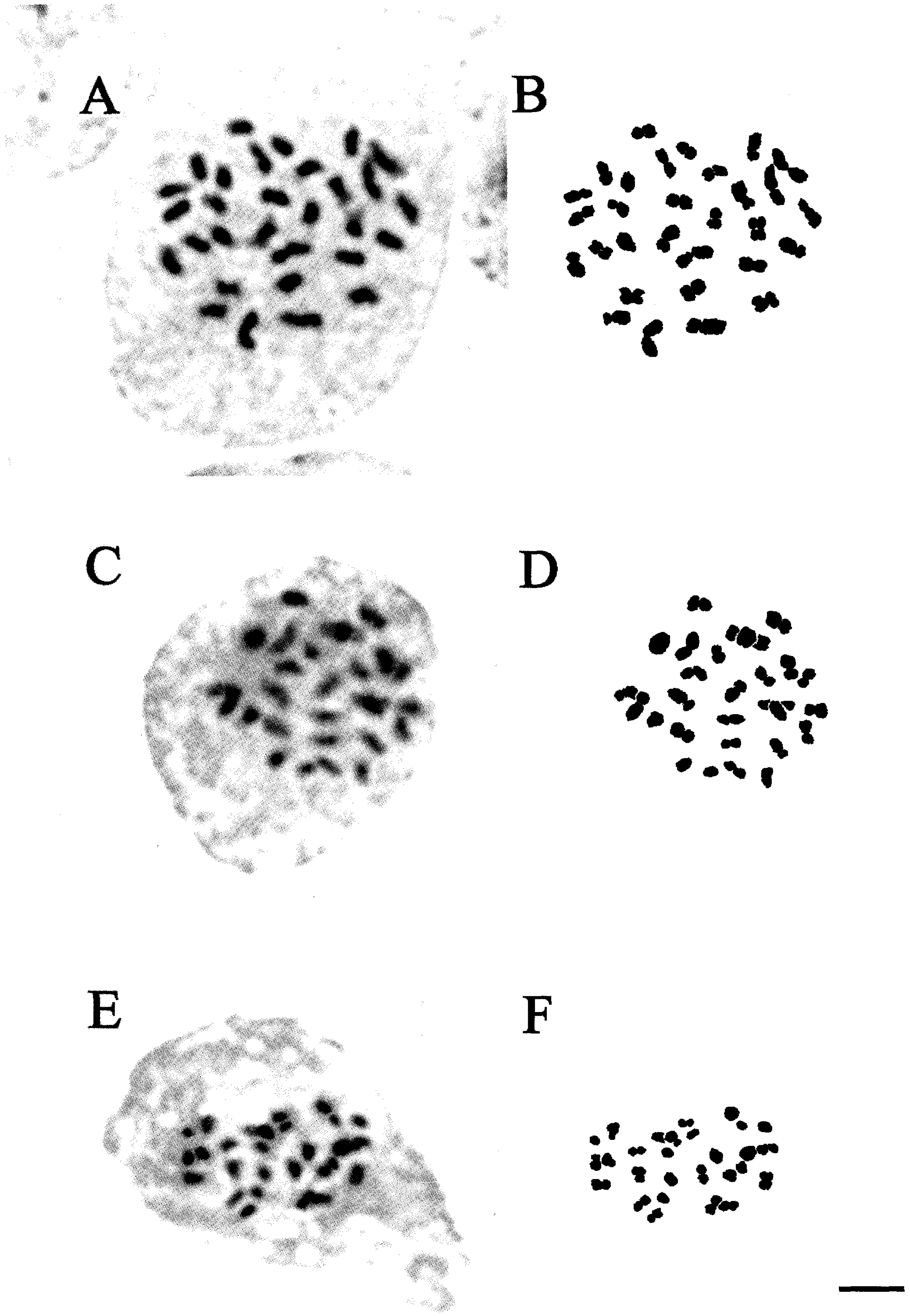

FIG. 1. Somatic chromosomes at metaphase in Irvingiaceae and Ixonanthaceae. A, C, E: micrographs. B, D, F: drawings of micrographs in A, C, and E, respectively. A, B: *Irvingia malayana* (2*n* = 30). C, D: *Ixonanthes icosandra* (2*n* = 28). E, F: *Ixonanthes reticulata* (2*n* = 28). Scale bar, 2 μm.

Lacistemataceae

The Lacistemataceae comprise 14 species in two genera, *Lacistema* Sw. and *Lozania* Mutis, which are distributed from central to southern South America (Takhtajan 2009). *Lacistema aggregatum* Rusby, which was studied here for the first time, had $2n = 44$. The chromosomes at metaphase gradually varied from about 0.9 to 2.3 μm (Fig. 2A, B) in length. Of the 44 chromosomes, most had a median or submedian centromere, although the centromere position in several small chromosomes was uncertain.

The base number of *Lacistema* is likely $x = 22$ (or 11). The Lacistemataceae are a sister group of Salicaceae (Davis *et al.* 2005, Tokuoka & Tobe 2006, Soltis *et al.* 2007, Wurdack & Davis 2009). The occurrence of $x = 11$ appears to be rare in other Malpighiales, but has been reported for some Salicaceae (e.g., *Flacourtia*, *Homalium*, and *Oncoba*) and some Euphorbiaceae *s.str.* (e.g., *Aleurites*, *Caperonia*, *Chrozophora*, *Ditta*, *Jatropha*, *Macaranga*, *Mallotus*, and *Suregada*). The base number of $x = 11$ may indicate affinities between Lacistemataceae and Salicaceae, although it occurs as a homoplasy in the Euphorbiaceae *s.str.* as well. This has yet to be confirmed with chromosome data from a basal-divergent clade in the family phylogeny.

Peraceae

The Peraceae comprise 135 species in the five genera *Chaetocarpus* Thwaites, *Clutia* L., *Pera* Mutis, *Pogonophora* Miers ex Benth., and *Trigonopleura* Hook.f., which have pantropical distributions (Stevens 2001 onwards). *Chaetocarpus castanocarpus* Thwaites, which was studied here for the first time, had $2n = 36$. Chromosomes at metaphase gradually varied from about 0.5 to 1.1 μm (Fig. 2C, D). Of the 36 chromosomes, most had a centromere at a median position, although their positions in several small chromosomes were uncertain.

The genera of the Peraceae are usually placed in the Euphorbiaceae *s.lat.* (e.g., Radcliffe-Smith 2001). However, recent molecular analyses have revealed that Rafflesiaceae are sister to Euphorbiaceae *s.str.*, excluding the genera of Peraceae, and that the Peraceae are sister to the clade of Rafflesiaceae and Euphorbiaceae *s.str.* (Wurdack & Davis 2009). Anatomically, the Peraceae are clearly distinguished from Euphorbiaceae *s.str.* (palisadal exotegmen) in having a tracheoidal exotegmen (Tokuoka & Tobe 2006). Given that *Chaetocarpus* has a base number of $x = 18$ (9), the Rafflesiaceae have $x = 11$ or 12, and Euphorbiaceae *s.str.* have diverse chromosome numbers such as $n = 8, 9, 10, 11, 12, 13$, the chromosome number of *Chaetocarpus* is unlikely to represent relationships with other families. To evaluate the evolutionary implications of these chromosome numbers, more information on the chromosomes of other genera and species of Peraceae is needed in addition to an analysis of the phylogenetic relationships within the family.

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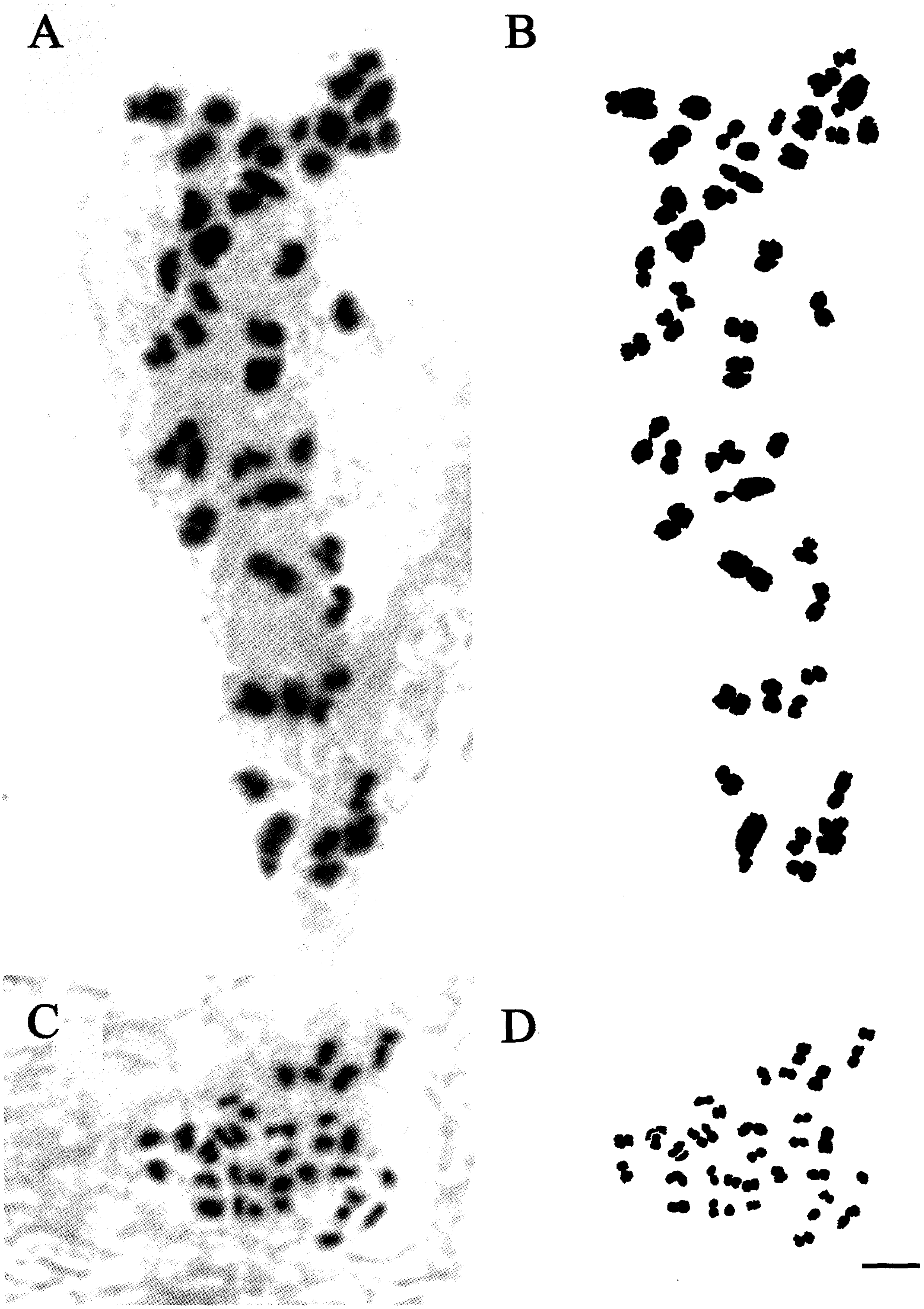

FIG. 2. Somatic chromosomes at metaphase in Lacistemataceae and Peraceae. A, C: micrographs. B, D: drawings of micrographs in A and C, respectively. A, B: *Lacistema aggregatum* ($2n = 44$). C, D: *Chaetocarpus castanocarpus* ($2n = 36$). Scale bar, 2 μm.

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